510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
K120625

B. Purpose for Submission:
Clearance of new device

C. Measurand:
Herpes Simplex Virus -1 (HSV-1) IgG antibodies to the glycoprotein G (gG) 1 recombinant antigen

D. Type of Test:
Electrochemiluminescence assay (ECLIA)

E. Applicant:
Roche Diagnostics

F. Proprietary and Established Names:
Proprietary Names: Elecsys HSV-1 IgG Immunoassay and PreciControl HSV
Common Names: HSV-1 IgG and PreciControl HSV

G. Regulatory Information:

1. Regulation section:
   21 CFR 866.3305, Herpes simplex virus serological assays
   21 CFR 862.1660, Quality control material (assayed and unassayed)

2. Classification:
   Class II

3. Product code:
   MXJ - Enzyme Linked Immunosorbent Assay, Herpes Simplex Virus, HSV-1
   JJX - Single (specified) analyte controls (assayed and unassayed)

4. Panel:
   Microbiology (83)

H. Intended Use:

1. Intended use(s):

   Elecsys HSV-1 IgG Immunoassay:
   The Roche Elecsys HSV-1 IgG immunoassay is a test for the *in vitro* qualitative

determination of IgG class antibodies to HSV-1 in human serum and lithium-heparin plasma, K_2 –EDTA plasma, and K_3 –EDTA plasma. The test is intended for sexually active individuals and expectant mothers as an aid in the presumptive diagnosis of HSV-1 infection. The predictive value of positive and negative results depends on the population's prevalence and the pretest likelihood of HSV-1.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

The test is not FDA cleared for screening blood or plasma donors.

The performance of this assay has not been established for use in a pediatric population, neonates and immunocompromised patients or for use at point of care facilities.

PreciControl HSV:
PreciControl HSV is used for quality control of the Elecsys HSV-1 IgG immunoassay on the Elecsys and **cobas e** immunoassay analyzers.

2. Indication(s) for use:
   Same as intended use.

3. Special conditions for use statement(s):
   For prescription use only

4. Special instrument requirements:
   Roche cobas e 411 and cobas e 601 immunoassay analyzers.

I. Device Description:

HSV-1 IgG Immunoassay:
The HSV-1 IgG is a two-step sandwich immunoassay with streptavidin microparticles, biotinylated recombinant HSV-1-specific antigen labeled with ruthenium complex and electrochemiluminescence detection. This assay is a qualitative test based on a cut-off formula dependent on the negative and positive calibrators. Cut-off index (COI) is based on the ratio of assay signal to cut-off signal (also abbreviated s/co). COI values greater than or equal to 1.0 are considered positive for the presence of anti-HSV-1 IgG antibody. Results are determined using a two-point calibration. The test system contains the human serum-based calibrators intended for use with the system.

PreciControl HSV:
The PreciControl HSV consists of two controls that are used for monitoring the accuracy of the HSV-1 IgG immunoassay. This product is manufactured as two bottles of lyophilized human serum with added HSV-1 IgG in two different

concentration ranges: human serum negative for HSV-1 IgG (target value = 0.30 COI) and human serum positive to HSV-1 IgG (target value = 4.00 COI).

Note: The reagents and calibrator are packaged together in the HSV-1 IgG immunoassay, while the associated PreciControl is packaged separately.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Focus HerpeSelect® 1 and 2 Immunoblot IgG

2. Predicate 510(k) number(s):
   K000238

3. Comparison with predicate:

Similarities		
Item	**HSV-1 IgG Immunoassay and PreciControl HSV**	**Focus HerpeSelect 1 and 2 Immunoblot IgG (Predicate)**
Intended Use	The Roche HSV-1 IgG immunoassay is a test for the *in vitro* qualitative determination of IgG class antibodies to HSV-1 in human serum and lithium-heparin plasma, K_2–EDTA plasma, and K_3–EDTA plasma. The test is intended for sexually active individuals and expectant mothers as an aid in the presumptive diagnosis of HSV-1 infection. The predictive value of positive and negative results depends on the population's prevalence and the pretest likelihood of HSV-1. The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on Elecsys	Focus Diagnostics' HerpeSelect 1 and 2 Immunoblot IgG test is intended for qualitatively detecting the presence or absence of human IgG class antibodies to HSV-1 and HSV-2 in human sera. The test is indicated for testing sexually active adults or expectant mothers for aiding in the presumptive diagnosis of HSV-1 and HSV-2 infection. The predictive value of a positive or negative result depends on the population's prevalence and the pretest likelihood of HSV-1 and HSV-2 infection. The performance of this assay has not been established for use in a

	and **cobas e** immunoassay analyzers. The test is not FDA cleared for screening blood or plasma donors. The performance of this assay has not been established for use in a pediatric population, neonates and immunocompromised patients or for use at point of care facilities.	pediatric population, for neonatal screening, for testing of immunocompromised patients, for use by a point of care facility or for use with automated equipment.
Sample Volume	20 µL	20 µL

Differences		
Item	Device	Predicate
Type of Assay	Two-step sandwich immunoassay	Nitrocellulose immunoblot
Measurement System	Electrochemiluminescent Immunoassay	Alkaline phosphatase (qualitative)
Platform	Elecsys 2010, MODULAR ANALYTICS E170, **cobas e** 411, **cobas e** 601, and **cobas e** 602	Manual procedure
Sample Matrix	Human serum and Lithium-heparin, K_2-EDTA, and K_3-EDTA plasma	Human serum
Reagents	Reagents consist of streptavidin-coated microparticles, biotinylated HSV-1 antigen (recombinant, from *E. coli*), ruthenylated HSV-1 antigen, and negative and positive calibrators.	Reagents consist of HSV-1 and HSV-2 differentiation antigen strips, alkaline phosphatase-conjugated goat anti-human IgG, bromo-chloro-indodyl phosphate and nitroblue tetrazolium substrate and negative and positive controls.
Calibrator	Included with the reagent kit	Not included

Differences		
Item	Device	Predicate
Controls	PreciControl HSV (not included with the kit)	Negative and positive controls (included with the kit)
Unit of Measure	Cutoff index (COI)	Positive or negative reading
Cutoff	The analyzer automatically calculates the cutoff based on the measurement of Cal 1 and Cal 2. The result of a sample is given either as reactive or non-reactive as well as in the form of a cutoff index (COI). For the HSV-1 IgG immunoassay, the interpretation of the results is: Non-reactive < 1.0 COI Reactive ≥ 1.0 COI	Positive or negative results are generated by this qualitative assay by comparing bands on the nitrocellulose to a cut-off/control strip.
Total Incubation	18 minutes	2 hours 20 minutes

K. Standard/Guidance Document Referenced (if applicable):

1. CLSI EP17-A – Protocols for Determination of Limits of Detection and Limits of Quantitation

2. CLSI EP5-A2 – User Verification of Performance for Precision and Trueness

3. CLSI EP15-A2 – User Verification of Performance for Precision and Trueness

L. Test Principle:

The HSV-1 IgG immunoassay is a two-step sandwich electrochemiluminsecent immunoassay. During the first step, samples are incubated with biotinylated HSV-1-specific recombinant antigens (*E. coli)* labeled with a ruthenium complex: Tris (2,2'-bipyridyl)ruthenium(II)-complex. If anti-HSV-1 IgG is present in the samples, it will bind to the ruthenium-labeled complex (HSV-1/IgG complex). In the second step, streptavidin-coated microparticles are added. These will bind to the biotin in the HSV-1/IgG complex. In the third step, the reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of

the electrode. Any unbound substances are removed with the ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. Results are determined automatically by the software by comparing the electrochemiluminescence signal obtained from the reaction product of the sample with the signal of the cutoff value previously obtained by the HSV-1 IgG calibration.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Precision of the HSV-1 IgG immunoassay was evaluated on the cobas e 411 and cobas e 601 analyzers. Reproducibility was evaluated on the cobas e 411 analyzer. Both assays used Elecsys reagents and were conducted according to CLSI EP5-A2 and CLSI EP15-A2 for precision and reproducibility, respectively.

For precision, five human sera (high positive, low positive, and moderate positive) and two control sera (PreciControl 1 and PreciControl 2) were tested in duplicate, two runs per day for 21 days with one reagent lot with calibration according to the instructions for use. The results of these studies are presented below.

Precision on cobas e 411

Table 1. Precision Results Summary: cobas e 411 Immunoassay Analyzer

		Repeatability		**Intermediate precision**	
Sample	Mean (COI)	SD (COI)	CV (%)	SD (COI)	CV (%)
PC[1] 1	0.24	0.003	1.2	0.006	2.5
PC 2	4.01	0.040	1.0	0.100	2.5
HS[2] 1	0.03	0.001	1.9	0.001	2.7
HS 2	0.87	0.011	1.3	0.025	2.9
HS 3	1.54	0.015	1.0	0.043	2.8
HS 4	5.47	0.072	1.3	0.153	2.8
HS 5	0.47	0.006	1.3	0.012	2.6

[1] PC = PreciControl
[2] HS = Human Serum

Precision on cobas e 601

Table 2. Precision Results Summary: cobas e 601 Immunoassay Analyzer

Sample	Mean (COI)	Repeatability		Intermediate precision	
		SD (COI)	CV (%)	SD (COI)	CV (%)
PC 1	0.24	0.003	1.2	0.005	2.0
PC 2	4.02	0.053	1.3	0.089	2.2
HS 1	0.02	0.000	1.4	0.000	1.6
HS 2	0.85	0.012	1.4	0.019	2.2
HS 3	1.51	0.020	1.3	0.031	2.1
HS 4	6.28	0.101	1.6	0.140	2.2
HS 5	0.47	0.008	1.6	0.010	2.2

For reproducibility, eight human sera (high negative, moderate positive, low positive and high positive) and two control sera (PreciControl 1 and PreciControl 2) were tested in triplicate, two runs per day for 5 days at three different sites. The results of these studies are presented below.

Reproducibility at Clinical Site 1

Table 3. HSV-1 IgG Immunoassay within Site Reproducibility on the cobas e 411 Analyzer at Clinical Site 1

			Repeatability		Between Run		Between Day		Within Site Reproducibility	
Sample	N	Mean COI[a]	SD[b]	% CV	SD	% CV	SD	% CV	SD	% CV
HSP 01	30	0.453	0.007	1.6	0.003	0.8	0.000[c]	0.0	0.008	1.8
HSP 02	30	0.654	0.012	1.8	0.006	1.0	0.000[c]	0.0	0.013	2.0
HSP 03	30	0.993	0.015	1.5	0.011	1.1	0.000[c]	0.0	0.019	1.9
HSP 04	30	1.636	0.032	1.9	0.018	1.1	0.000[c]	0.0	0.036	2.2
HSP 05	30	3.706	0.051	1.4	0.049	1.3	0.000[c]	0.0	0.070	1.9
HSP 06	30	4.702	0.075	1.6	0.026	0.6	0.000[c]	0.0	0.080	1.7
HSP 07	30	14.380	0.319	2.2	0.221	1.5	0.000[c]	0.0	0.388	2.7
HSP 08	30	30.197	0.552	1.8	0.660	2.2	0.000[c]	0.0	0.860	2.8
PC HSV 1	30	0.238	0.004	1.5	0.001	0.4	0.000[c]	0.2	0.004	1.6
PC HSV 2	30	3.758	0.053	1.4	0.035	0.9	0.000[c]	0.0	0.063	1.7

[a] COI - Cutoff index
[b] SD - Standard deviation
[c] SD of zero due to variance contributed by particular component was below stated significant figure.

Reproducibility at Clinical Site 2

Table 4. HSV-1 IgG Immunoassay within Site Reproducibility on the cobas e 411 Analyzer at Clinical Site 2

			Repeatability		Between Run		Between Day		Within Site Reproducibility	
Sample	N	Mean COI[a]	SD[b]	% CV	SD	% CV	SD	% CV	SD	% CV
HSP 01	30	0.469	0.005	1.0	0.022	4.7	0.000[c]	0.0	0.022	4.8
HSP 02	30	0.676	0.018	2.7	0.037	5.5	0.000[c]	0.0	0.041	6.1
HSP 03	30	1.011	0.020	2.0	0.037	3.6	0.000[c]	0.0	0.042	4.2
HSP 04	30	1.680	0.035	2.1	0.086	5.1	0.000[c]	0.0	0.093	5.6
HSP 05	30	3.795	0.080	2.1	0.201	5.3	0.000[c]	0.0	0.216	5.7
HSP 06	30	4.888	0.094	1.9	0.297	6.1	0.000[c]	0.0	0.312	6.4
HSP 07	30	14.786	0.239	1.6	0.669	4.5	0.000[c]	0.0	0.710	4.8
HSP 08	30	30.743	0.650	2.1	1.319	4.3	0.000[c]	0.0	1.471	4.8
PC HSV 1	30	0.251	0.006	2.5	0.014	5.5	0.000[c]	0.0	0.015	6.0
PC HSV 2	30	3.856	0.097	2.5	0.208	5.4	0.000[c]	0.0	0.230	6.0

[a] COI - Cutoff index
[b] SD - Standard deviation
[c] SD of zero due to variance contributed by particular component was below stated significant figure.

Reproducibility at Clinical Site 3

Table 5. HSV-1 IgG Immunoassay within Site Reproducibility on the cobas e 411 Analyzer at Clinical Site 3

			Repeatability		Between Run		Between Day		Within Site Reproducibility	
Sample	N	Mean COI[a]	SD[b]	% CV	SD	% CV	SD	% CV	SD	% CV
HSP 01	30	0.438	0.003	0.8	0.014	3.1	0.000[c]	0.0	0.014	3.2
HSP 02	30	0.634	0.011	1.7	0.010	1.6	0.012	1.9	0.019	3.0
HSP 03	30	0.963	0.014	1.5	0.026	2.7	0.000[c]	0.0	0.030	3.1
HSP 04	30	1.570	0.031	2.0	0.050	3.2	0.054	3.4	0.079	5.1
HSP 05	30	3.624	0.033	0.9	0.075	2.1	0.054	1.5	0.098	2.7
HSP 06	30	4.630	0.066	1.4	0.039	0.8	0.076	1.6	0.108	2.3
HSP 07	30	14.162	0.294	2.1	0.141	1.0	0.067	0.5	0.333	2.4
HSP 08	30	28.354	0.723	2.6	0.415	1.5	0.941	3.3	1.257	4.4
PC HSV 1	30	0.229	0.003	1.3	0.001	0.6	0.004	1.7	0.005	2.2
PC HSV 2	30	3.578	0.070	2.0	0.078	2.2	0.099	2.8	0.144	4.0

[a] COI - Cutoff index
[b] SD - Standard deviation
[c] SD of zero due to variance contributed by particular component was below stated significant figure.

Reproducibility between sites

Table 6. HSV-1 IgG Immunoassay between Site Reproducibility on the cobas e 411 Analyzer for All Sites

			Repeatability		Between Run		Between Day		Between Site		Reproducibility	
Sample	N	Mean COI[a]	SD[b]	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV
HSP 01	90	0.453	0.005	1.2	0.015	3.3	0.000[c]	0.0	0.015	3.4	0.022	4.9
HSP 02	90	0.655	0.014	2.1	0.022	3.4	0.000[c]	0.0	0.020	3.1	0.033	5.1
HSP 03	90	0.989	0.017	1.7	0.027	2.7	0.000[c]	0.0	0.023	2.3	0.039	3.9
HSP 04	90	1.629	0.033	2.0	0.058	3.6	0.019	1.2	0.051	3.1	0.086	5.3
HSP 05	90	3.708	0.058	1.6	0.127	3.4	0.000[c]	0.0	0.077	2.1	0.159	4.3
HSP 06	90	4.740	0.079	1.7	0.174	3.7	0.000[c]	0.0	0.127	2.7	0.229	4.8
HSP 07	90	14.443	0.286	2.0	0.415	2.9	0.000[c]	0.0	0.299	2.1	0.586	4.1
HSP 08	90	29.764	0.645	2.2	0.885	3.0	0.262	0.9	1.209	4.1	1.652	5.6
PC HSV 1	90	0.240	0.005	1.9	0.008	3.3	0.000[c]	0.0	0.011	4.5	0.014	5.9
PC HSV 2	90	3.731	0.076	2.0	0.130	3.5	0.000[c]	0.0	0.135	3.6	0.202	5.4

[a] COI - Cutoff index
[b] SD - Standard deviation
[c] SD of zero due to variance contributed by particular component was below stated significant figure.

b. Linearity/assay reportable range:

Not applicable.

c. Traceability, Stability, Expected values (controls, calibrators, or methods)

Traceability: There is no international standard available for measuring HSV-1 antibody in serum, therefore an internal Roche HSV reference standard was prepared from high positive serum.

Controls: The two controls in the PreciControl HSV (PC1: 0.25 COI and PC2: 4.0 COI) consist of a buffer-based/human serum matrix with a pH of 7.4.

Calibrators: Calibrators are manufactured with human sera negative for HSV-1 IgG (negative calibrator; CS1: 0.15 U/mL) or positive for HSV-1 IgG (positive calibrator; CS2: 5.0 U/mL). The two calibrators are part of the HSV-1 IgG immunoassay kit and they consist of a buffer-based/human serum matrix with a pH of 7.4.

Stability:

Reagents:

After opening at 2-8 °C	Up to 13 weeks
Opened and stored on board the cobas e 411 or cobas e 601 (at 20 °C ± 3 °C)	Up to 3 weeks

Calibrators:

After opening at 2-8 °C	Up to 3 weeks
After opening at 25 °C	Up to 6 hours on the cobas e 411 & 3 hours on the cobas e 601
After opening at 32 °C	Up to 5 hours on the cobas e 411 & 2 hours on the cobas e 601

PreciControl:

After opening at 2-8 °C	Up to 3 weeks
After opening at 25 °C	Up to 6 hours on the cobas e 411 & 3 hours on the cobas e 601
After opening at 32 °C	Up to 5 hours on the cobas e 411 & 3 hours on the cobas e 601
After opening at 2-8 °C	Up to 16 months

Samples: Sample stability was determined using a panel of 10 native human serum samples (high negative, moderately positive, low positive and high positive) and two control sera (PreciControl 1 and PreciControl 2). Samples were measured directly after collection (for reference values) and then measured again after storage under the four conditions listed below to determine stability.

Stored at 2-8 °C	Up to 48 hours
Stored at room temperature (20-25 °C)	Up to 9 hours
Stored at -20 °C	Up to 21 weeks
After freeze/thaw cycles	Up to 6 cycles

d. Detection limit:

Not applicable.

e. Analytical specificity:

Cross-reactivity:

129 samples from individuals with IgG antibodies for 13 potentially cross-reactive analytes capable of producing similar clinical symptoms (ANA, *Candida*

albicans, *Chlamydia trachomatis*, CMV, *E. coli*, EBV, HIV, HSV-2, *Neisseria gonorrhea*, Rubella, *Treponema pallidum*, *Toxoplasma gondii*, and VZV) were tested in duplicate in an analytical specificity study on the cobas e 411 analyzer. All 129 samples were found to be nonreactive (negative) in both the HSV-1 IgG immunoassay and the predicate assay, Focus HerpeSelect 1 Immunoblot IgG. The results of this study are summarized in the table below:

Table 7. Cross Reactivity on the HSV-1 IgG Immunoassay

Cross-reactant	No. Tested	HSV-1 IgG immunoassay/Reference Negative/Negative	HSV-1 IgG immunoassay/Reference Positive/Positive
ANA	9	9	0
Candida albicans	6	6	0
Chlamydia trachomatis	10	10	0
CMV	15	15	0
E. Coli	5	5	0
EBV	9	9	0
HIV	9	9	0
HSV-2	22	22	0
Neisseria gonorrhea	4	4	0
Rubella	10	10	0
Treponema pallidum (Syphilis)	18	18	0
Toxoplasma gondii	4	4	0
VZV	8	8	0
Total	129	129	0

Interference (Endogenous substances):

The impact of endogenous interfering substances on the HSV-1 IgG immunoassay was determined by testing natural and spiked serum samples. Spiked serum samples were created by diluting the serum with the interfering substance in 10% increments. The following substances were evaluated in three pools of human serum samples (negative for HSV-1 IgG, positive for HSV-1 IgG, and near the cut-off):

The level of each potentially interfering substance is as follows:
Hemoglobin: 100 mg/dL (low), 1000 mg/dL (high)
Biotin: 7.0 ng/mL (low), 70 ng/mL (high)
Intralipid®: 200 mg/dL (low), 2000 mg/dL (high)
Bilirubin: 6.6 mg/dL (low), 66.0 mg/dL (high)
Rheumatoid Factor (RF): 10 IU/mL (low), 1500 IU/mL (high)

The potentially interfering endogenous substances were added to the samples ("spiked sample") and then samples were evaluated in duplicate. The percent recovery COI was determined for each spiked sample and compared to the reference ("unspiked") sample. The % recovery was determined by dividing the mean value of the measured concentration by the expected concentration. The acceptance criterion was a recovery of positive samples within ± 20% of the initial value

All positive and near cut-off samples showed a change of signal less than 5% for all interfering substances except the near cut-off sample spiked with RF. Near cut-off samples spiked with RF showed a dose-dependent increase in the % recovery (maximum % recovery was 107.3 at the 1500 IU/mL dose). All positive samples remained positive. No sample went from positive to negative or negative to positive. Negative samples showed a deviation in COI that was ≤ 0.01 for all interfering substances except RF. Negative samples spiked with RF showed a dose-dependent increase in COI deviation. The maximum COI deviation was 0.05 at the 1500 IU/mL dose. No interference was produced in the assay by the presence of hemoglobin, biotin, Intralipid®, and bilirubin.

Interference (Drugs):

A total of 21 drugs were tested for interference with the HSV-1 IgG immunoassay. Samples were created by adding drug into human serum samples. All drugs were evaluated in three pools of human serum samples (negative for HSV-1 IgG, positive for HSV-1 IgG, and near the cut-off). The drugs used and the concentration at which they were tested are listed below:

Compound	**Concentration**
Acetylcysteine	150 mg/L
Ampicillin-Na	1000 mg/ L
Ascorbic acid	300 mg/ L
Ca- Dobesilate	200 mg/L
Cyclosporine	5 mg/L
Cefoxitin	2500 mg/L
Heparin	5000 U
Intralipid	10000 mg/L
Levodopa	20 mg/L

	Methyldopa + 1.5 H_2O	20mg/L
	Metronidazole	200 mg/L
	Phenylbutazone	400 mg/L
	Doxycycline	50 mg/L
	Acetylsalicylic Acid	1000 mg/L
	Rifampicin	60 mg/L
Drugs	Acetaminophen	200 mg/L
samples	Ibuprofen	500 mg/L
and then	Theophylline	100 mg/L
	Special Drugs	
	Aciclovir	1.2 mg/L
recovery	Famvir	0.25 mg/L
for each	Valaciclovir	3 mg/L

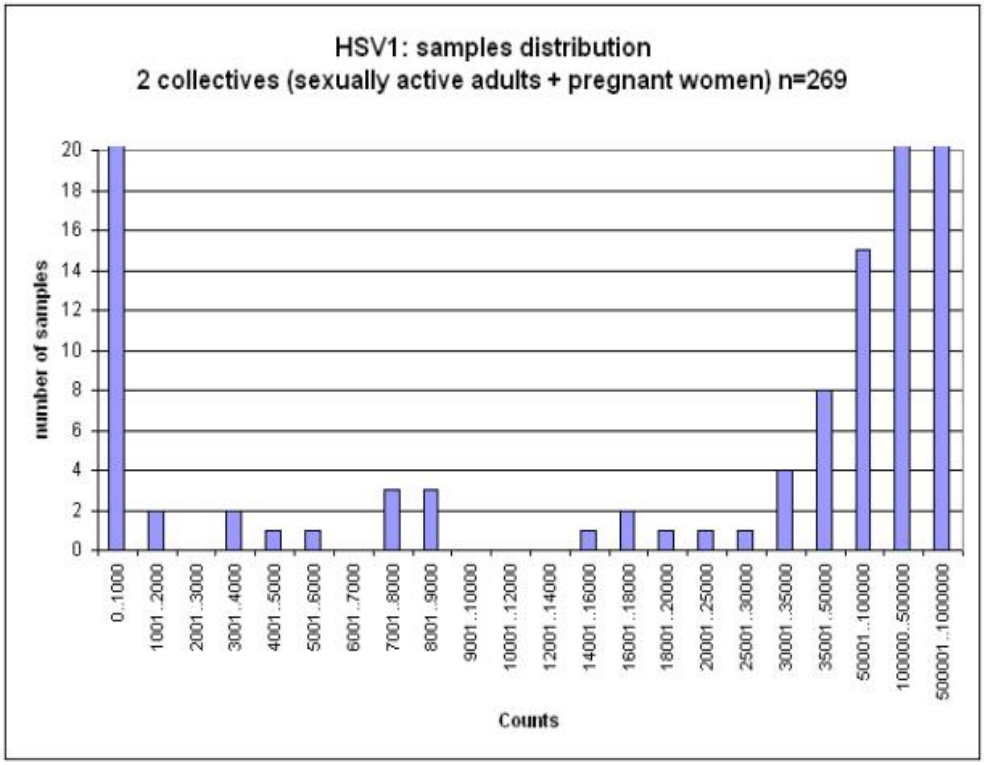
were added to the ("spiked sample") samples were evaluated in triplicate. The percent COI was determined spiked sample and compared to the reference ("unspiked") sample. The % recovery was determined by dividing the mean value of the measured concentration by the expected concentration. The acceptance criterion was a recovery of positive samples within ± 3 SD or ± 10% (whichever is a tighter criterion) of the value for the unspiked (reference) sample.

Negative samples spiked with drug did not show a change in COI compared to the reference. All near cut off samples showed a change in signal ≤ 0.05 COI compared to the unspiked reference. In the near cut off samples, the maximum COI deviation occurred in the samples spiked with Cefoxitin (-0.03 COI) and Acetylsalicylic Acid (-0.04 COI). All positive samples showed a change in signal ≤ 0.10 COI compared to the unspiked reference. In the positive samples, the maximum COI deviation occurred in the samples spiked with Cefoxitin (-0.43 COI), Phenylbutazone (+0.11 COI), and Acetylsalicylic Acid (-0.15 COI). These results met the acceptance criteria as the recovery of the spiked samples was within 10% of the value for the reference sample (reference sample for all three was 7.13 COI therefore the acceptance criterion was ± 0.713).

f. Assay cut-off:

The cut-off for the HSV-1 IgG immunoassay was initially established by measuring 269 native human sera samples from two cohorts: sexually active adults and pregnant women. The distribution of negative and positive samples was determined on the cobas e 411 immunoassay analyzer using lab lot reagents. The results from this study are summarized in the graph below.

Figure 1. Sample Distribution for the HSV-1 IgG Immunoassay



These results were then compared to the predicate device, the Focus HerpeSelect IgG Immunoblot. Discrepant positives (false positives) and discrepant negatives (false negatives) were compared in the two cohorts and summarized in the following table.

Table 8. Comparison to the Predicate Device

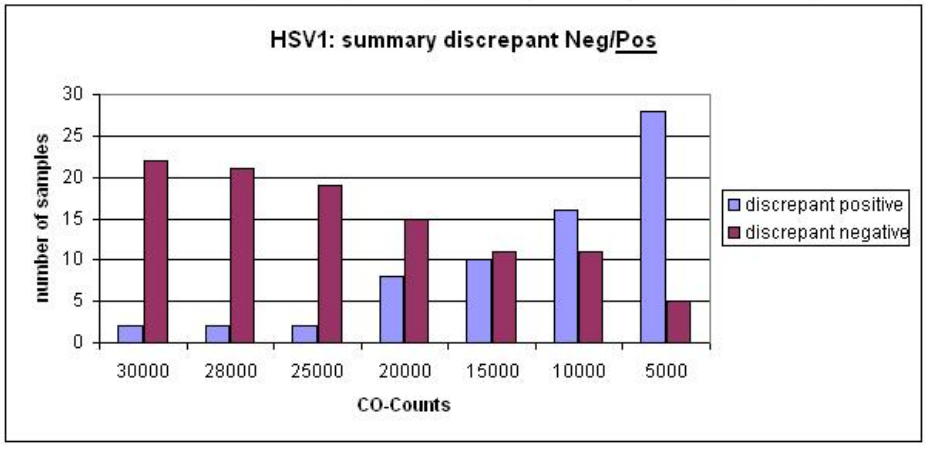
	CO-Counts	**30000**	**25000**	**20000**	**15000**	**10000**	**5000**
Sexually active	Discr. Neg	0	0	0	0	0	0
	Discr. Pos	2	2	2	2	2	2
Pregnant	Discr. Neg	7	5	5	3	3	0
	Discr. Pos	1	3	3	5	5	3
Total discrepant negative		**7**	**5**	**5**	**3**	**3**	**0**
Total discrepant positive		**3**	**5**	**5**	**7**	**7**	**11**

Based on these results, the cut-off was set between 25,000 and 30,000 counts. This was selected in an effort to minimize the number of false positives.

The cut-off was verified/challenged using a selection of 85 near cut-off samples (taken from 1248 commercially available native human sera) that were tested with the Focus HerpeSelect IgG Immunoblot and the HSV-1 IgG immunoassay. The percent positive agreement and the percent negative agreement were determined for various cut-off levels. They are reported in the table below.

Figure 2. Agreement of Negative and Positive Results in Samples Near the Cut-Off

CO-Counts	30000	28000	25000	20000	15000	10000	5000
discrepant negative	22	21	19	15	11	11	5
discrepant positive	2	2	2	8	10	16	28
overall agreement (%)	72	73	75	73	75	68	61
negative agreement (%)	95	95	95	78	73	57	24
positive agreement (%)	54	56	60	69	77	77	90



A cut-off of 28,000 was selected as the percent negative agreement was 95% and due to a very low number of false positives.

2. Comparison studies:

a. Method comparison with reference method:

The performance of the HSV-1 IgG immunoassay was evaluated in a clinical study by comparing the subject device against a reference method, FDA-cleared immunoblot. When samples repeatedly tested equivocal in the reference method, a confirmatory method, the University of Washington Western blot, was used to resolve these results, as per the reference method labeling instructions. Any remaining equivocal or discordant result was counted against the HSV-1 IgG immunoassay in the performance analysis.

b. Matrix comparison:

The effect of anticoagulants on the detection of analyte in HSV-1 IgG immunoassay was determined on the cobas e 411 analyzer by comparing values obtained from human serum samples drawn into serum, lithium-heparin plasma, K_2-EDTA-Plasma, K_3-EDTA-Plasma, and serum-gel separation primary tubes. At least 35 serum/plasma pairs were tested for each of the anticoagulants. For each anticoagulant, three serum concentrations were tested (negative, near cut-off, and positive). Each sample was tested in triplicate. The acceptance criterion was a recovery of positive plasma samples within ± 20% of the serum reference value (serum drawn into primary tubes without gel). All anticoagulant –treated plasma samples met this criterion. The results of this study are displayed in the summary table below.

Table 9. Matrix Comparison Studies with the HSV-1 IgG Immunoassay

Plasma matrix	Number of Positive Specimens Showing Recovery to Serum within Various Ranges		
	< 10 %	10-20 %	> 20 %
Li-heparin	28	0	0
K2-EDTA	28	0	0
K3-EDTA	26	2	0
Serum separation tube	30	0	0
Plasma matrix	Number of Negative Specimens Showing Recovery to Serum within Various Ranges		
	< 10 %	10-20 %	> 20 %
Li-heparin	6	1	0
K2-EDTA	7	0	0
K3-EDTA	7	0	0
Serum separation tube	10	0	0

3. Clinical studies:

a. Clinical Sensitivity: Not applicable.

b. Clinical specificity: Not applicable.

c. Other clinical supportive data (when a. and b. are not applicable):

Clinical study populations:

A multi-center study was conducted in the U.S. to characterize the performance of HSV-1 IgG immunoassay. All subjects were tested with the HSV-1 immunoassay on the cobas e 411 analyzer and with an acceptable reference method.

All samples were collected prospectively at the specimen collection sites and then stored frozen before shipment to Roche. Upon arrival at Roche, samples were divided into aliquots, refrozen, and then shipped to the study sites for testing. All testing was performed using a sample which had been frozen and thawed once. Among the samples, 325 (40.6%) were females and 475 (59.4%) were males. Ages ranged from 16 to 90 years. All female subjects were tested for pregnancy at a single site using the Roche human chorionic gonadotropin (hCG) +beta assay on the Elecsys 2010 analyzer.

A total of 800 samples were obtained from multiple specimen sources, representing subjects for whom HSV-1 testing would be ordered and a low prevalence group. These populations included: sexually active individuals with an HSV-1 IgG and/or HSV-2 IgG test ordered (n = 600) and low prevalence population (n = 200). Out of the 600 samples collected from the sexually active group, 125 samples were identified as pregnant women. The specimens were tested with both the reference method and with the HSV-1 IgG immunoassay on the cobas e 411 analyzer.

Samples that repeatedly tested equivocal on the predicate device (n = 13) were resolved using a validated Western blot reference test (University of Washington, Seattle, WA), as per the instructions of the predicate device package insert. One sample in the sexually active cohort remained unresolved after testing with Western blot. This sample was scored as discrepant against the HSV-1 IgG immunoassay (see Table 8.3.6.7 below).

Table 10. Performance of Expectant Mothers Population

		Reference Method			
		Positive	**Equivocal**	**Negative**	**Total**
HSV-1 IgG Immunoassay	**Positive**	71	0	2	73
	Negative	7	0	45	52
	Total	78	0	47	125

Agreement classification	**Numerator / Denominator**	**Percent Agreement**	**95% Confidence Interval**
Specificity	45/47	95.7	85.5-99.5
Sensitivity	71/78	91.0	82.4-96.3

Table 11. Performance of the Sexually Active Adults Population

		Focus Immunoblot HSV-1 IgG +Western Blot			
		Positive	**Equivocal**	**Negative**	**Total**
HSV-1 IgG Immunoassay	**Positive**	341	0	23	364
	Negative	20	1	215	236
	Total	361	1	238	600

Agreement classification	**Numerator / Denominator**	**Percent Agreement**	**95% Confidence Interval**
Specificity	215/238	90.34	85.85-93.77
Sensitivity	341/362	94.20	91.27-96.37

Agreement with the CDC Panel

A panel of serum samples (n = 100) was obtained from the U.S. Centers for Disease Control and Prevention (CDC) and tested for confirmatory purposes. The CDC sample panel was tested on the HSV-1 IgG immunoassay on the cobas e 411 analyzer and then results were sent to the CDC for evaluation. The panel consisted of 52 samples negative for HSV-1 IgG and 48 samples positive for HSV-1 IgG. The HSV-1 IgG immunoassay demonstrated 100% positive agreement (48/48) and 100% negative agreement (52/52) with the results from the CDC.

Table 12. Performance of Low Prevalence Population

		Focus Immunoblot HSV-1-IgG +Western Blot			
		Positive	**Equivocal**	**Negative**	**Total**
HSV-1 IgG Immunoassay	**Positive**	74	0	4	78
	Negative	4	0	118	122
	Total	78	0	122	200

Agreement classification	**Numerator / Denominator**	**Percent Agreement**	**95% Confidence Interval**
Specificity	118/122	96.72	91.82-99.10
Sensitivity	74/78	94.87	87.39-98.59

4. Clinical cut-off**:**
   Please refer to assay cut-off section above for details.

5. Expected values/Reference range:
   The HSV-1 IgG immunoassay was used to evaluate the prevalence of HSV-1 IgG antibodies in expectant mothers and sexually active adults. The prospective study population for the HSV-1 IgG immunoassay consisted of 800 patients. Of these 800 subjects, 200 were low-risk individuals and 600 were sexually active individuals, with 125 of the sexually active individuals identified as a pregnant sub-cohort. The data for the intended use population (600 specimens) have been summarized according to age group in decades, gender, number of reactive results, and number of non-reactive results.

Table 13. Expected Results for HSV-1 IgG Immunoassay in Pregnant Subjects

Age range	Results from Elecsys HSV-1 IgG Immunoassay				Total
	Reactive		Non-reactive		
	N	Percent	N	Percent	
18 to 19	4	33.3	8	66.7	12
20 to 29	42	54.6	35	45.5	77
30 to 39	26	76.5	8	23.5	34
40 to 49	1	50.0	1	50.0	2
All ages	73	58.4	52	41.6	125
Total	73	58.4	52	41.6	125

Table 14. Expected Results for HSV-1 IgG Immunoassay in Sexually Active Subjects

Age range	Gender	Results from Elecsys HSV-1 IgG Immunoassay				Total
		Reactive		Non-reactive		
		N	Percent	N	Percent	
18 to 19	Male	11	47.8	12	52.2	23
	Female	3	60.0	2	40.0	5
20 to 29	Male	65	54.6	54	45.4	119
	Female	24	46.2	28	53.9	52
30 to 39	Male	44	65.7	23	34.3	67

	Female	8	38.1	13	61.9	21
40 to 49	Male	28	68.3	13	31.7	41
	Female	13	65.0	7	35.0	20
50 to 59	Male	22	71.0	9	29.0	31
	Female	19	70.4	8	29.6	27
60 to 69	Male	11	57.9	8	42.1	19
	Female	15	75.0	5	25.0	20
70 to 79	Male	9	81.8	2	18.2	11
	Female	10	100	0	0.00	10
80 to 89	Male	5	100	0	0.00	5
	Female	2	100	0	0.00	2
unknown	Male	1	100	0	0.00	1
	Female	1	100	0	0.00	1
All ages	Male	196	61.8	121	38.2	317
	Female	95	60.1	63	39.9	158
Total		291	61.3	184	38.7	475

The hypothetical predictive values for the two populations are shown in the table below. The calculations are based on the HSV-1 IgG immunoassay having:

1. Specificity of 90.70% and sensitivity of 93.97% in sexually active adults.
2. Specificity of 95.53% and sensitivity of 91.43% in pregnant women.

Table 15. Hypothetical Predictive Values

	HSV-1 IgG			
	Sexually Active Individuals		**Pregnant Women**	
Prevalence	**PPV**	**NPV**	**PPV**	**NPV**
50.00%	90.70%	93.97%	95.53%	91.43%
40.00%	86.66%	95.89%	93.45%	94.12%
30.00%	80.69%	97.32%	90.16%	96.14%
25.00%	76.47%	97.90%	87.70%	96.97%

	HSV-1 IgG			
	Sexually Active Individuals		**Pregnant Women**	
Prevalence	**PPV**	**NPV**	**PPV**	**NPV**
20.00%	70.90%	98.42%	84.25%	97.71%
15.00%	63.24%	98.88%	79.06%	98.37%
10.00%	51.99%	99.29%	70.39%	98.97%
5.00%	33.91%	99.66%	52.96%	99.51%

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.